

Fibres of Highly Oriented Pf1 Bacteriophage Produced in a Strong Magnetic Field

Highly oriented fibres of the cylindrical bacteriophage Pf1 have been produced by drying a concentrated solution in a magnetic field of 8 Tesla. Substantial improvement in the alignment of the phage parallel to the fibre axis is demonstrated by X-ray diffraction. This effect is due to the intrinsic diamagnetic anisotropy of the viruses and to their co-operative behaviour.

The molecular structure and anisotropic physical properties of polymers and elongated biological complexes can be studied most effectively in a highly oriented state. Alignment is often achieved by applying a mechanical stress by, for example, stretching, spinning or shearing. Magnetic fields have been used to orient retinal rod outer segments (Chalazonitis *et al.*, 1970; Saibil *et al.*, 1976), chloroplasts (Geacintov *et al.*, 1972), bacterial chromatophores (Clement-Metral, 1975) and purple membranes (Neugebauer *et al.*, 1977). Partially aligned fibres of Pf1 bacteriophage have been made by slowly drying concentrated solutions suspended between glass rods (Marvin *et al.*, 1974). Here we report that by carrying out the latter procedure in a strong magnetic field of 8 Tesla a significant improvement has been obtained in the X-ray fibre diffraction pattern from Pf1. We anticipate that a detailed description of the phage structure will be substantially aided by the use of magnetically aligned fibres.

Pf1 is a rod-like single-stranded DNA containing bacteriophage. It has a diameter of about 6 nm (Marvin & Wachtel, 1975,1976), a length of 1960 nm, and contains 7600 coat protein molecules and a similar number of DNA bases (Wiseman *et al.*, 1976; Wiseman & Day, 1977). At one end of the phage there are three to four copies of the absorption protein (Goldsmith & Konigsberg, 1977; Woolford *et al.*, 1977). Each coat protein is composed of 46 amino acids, which have been estimated by spectroscopic techniques to be about 100% α -helical (Day, 1969; Thomas & Murphy, 1975). From X-ray fibre diffraction patterns a model has been proposed in which the DNA is encapsulated in a helical protein shell made of 22 coat protein subunits in five turns of the helix (Marvin & Wachtel, 1975,1976). In this model the coat proteins are slightly curved α -helical rods which run almost parallel to the phage long axis and overlie radially, rather like scales in a fish. The formulation of a reliably detailed model is hindered because it is not possible, on the basis of the X-ray diffraction patterns available at present, to conclusively choose between the above preferred structure and another with 27 subunits in five turns. The choice is made more difficult because physical and chemical measurements on solutions strongly favour the latter structure (Wiseman *et al.*, 1976; Wiseman & Day, 1977). So far the X-ray work has given little information about the structure of the DNA (Marvin & Wachtel, 1975,1976).

The phage, prepared as described by Marvin *et al.* (1974), was a gift from Dr D. Marvin, European Molecular Biology Laboratory, Heidelberg. A volume of 10 μ l of a birefringent solution (\sim 20 mg/ml) in 0.01 M-Tris·HCl at pH7.5 was suspended over the 2 mm gap formed between two fine horizontal glass rods. These were placed in a horizontal Bitter magnet with their axes parallel to the field lines. In a constant field of

8 Tesla. (Tesla = 10^4 gauss), the solvent was evaporated off in a gentle stream of dry nitrogen. The time taken for fibre formation was less than 30 minutes.

In this way highly birefringent fibres were produced. These were X-rayed in the European Molecular Laboratory, Heidelberg, where the diffraction pattern in Figure 1 was recorded. This pattern is more detailed than those obtained previously (see Fig. 1 of Wachtel *et al.*, 1976), the layer-lines are clear and sharp with fine structure and in the low angle region the spots are better resolved than before. The best results in the past were obtained when fibre formation took place over about 24 hours (Marvin *et al.*, 1974). Further improvement may be possible if the procedure of slow drying at controlled relative humidities is carried out in a strong magnetic field.

We have found (the results will be reported in detail elsewhere): firstly, that the viruses are diamagnetically anisotropic due to their nearly axially oriented α -helices and aromatic amino acids; secondly, that above a critical concentration co-operative behaviour occurs probably due to long-range electrostatic repulsive forces. The latter

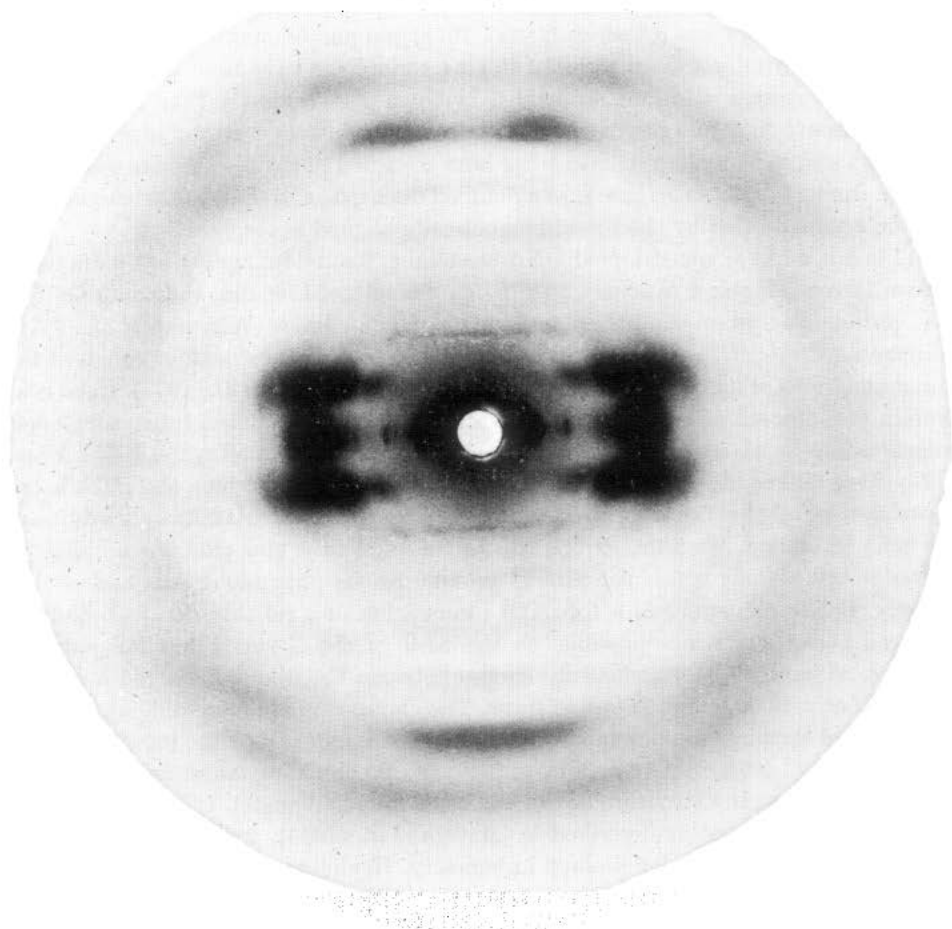


FIG. 1. X-ray fibre diffraction pattern of bacteriophage Pf1. This should be compared with Figure 1 of Wachtel *et al.* (1976).

phenomenon accounts for magnetically induced improvement in orientation in fibres and for the full alignment we have observed in solution.

Modest magnetic fields (<2 Tesla) have been used to orient purple membrane sheets (Neugebauer *et al.*, 1977) and retinal rod outer segments (Saibil *et al.*, 1976; Chabre, 1978). These are large structures which contain many parallel aligned α -helices (Worcester, 1978; Chabre, 1978). Pfl, which has about one tenth the α -helical content of a purple membrane sheet, can also be magnetically oriented in solution. Many other biological materials, such as collagen (Cotton-Feytis & Faure-Fremiet, 1942), muscle fibres (Arnold *et al.*, 1958) and DNA (Maret & Dransfeld, 1977), are magnetically anisotropic. Magnetic fields of about 10 Tesla are now easily obtainable and so magnetically induced or enhanced orientation is increasingly feasible. The magnetic anisotropy of an individual particle may not be large enough to give rise to useful orientation but if they associate in some way the effect could be greatly increased. Crystal growth might be tried in a magnetic field. There may also be some biochemical applications, such as in the reconstitution of filamentous complexes.

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